Pharmacological Functional MRI of *Psammomys Obesus* Brain During Melatonin Stimulation

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Introduction

Pharmacological functional magnetic resonance imaging (ph-MRI) is a non-invasive technique, which uses the changes in cerebral blood flow and the blood oxygen level dependent (BOLD) contrast to characterize pharmacological stimulation (1). Melatonin, the hormone produced and secreted nocturnally from the pineal gland (2) under the control of the biological clock in the suprachiasmatic nuclei, is a signal of darkness in the organism and as such it is an important regulatory component of the circadian timing system (biological clock). Melatonin induces sleep and is hypothermic in diurnal animals (humans) whereas in nocturnal animals (rodents) it is associated with wakefulness and has thermotropic activity (3).

This study examines the influence of melatonin injection stimulation in various brain areas of young and adult fat sand rat *Psammomys Obesus*. *Psammomys Obesus* is a diurnal desert rodent, which is distributed throughout North Africa, Arabia and Israel.

Recently, the development of circadian rhythmicity in the fat sand rat was found to adopt the diurnal activity pattern of their parents before weaning. Surprisingly, a few days after the weaning they become nocturnal and revert to diurnally again when mature. In this study we investigated for the first time the brain areas at which melatonin may exert these activities.

Methods

Young (N=4) and adult (N=5) *Psammomys Obesus* of 60-80g and 140-170g body weights were used, respectively. The animals were anaesthetized with 1 ml/kg ketamine and 0.34 ml/kg xylazine prior to the MRI experiments. The body temperature of the *Psammomys Obesus* was maintained at 37 ^{o}C throughout the duration of the experiment, using a circulating water blanket.

MRI experiments were performed on an 8.4 T spectrometer (Bruker, Germany) equipped with a minimaging accessory using a home built surface coil. The ph-MRI protocol included of a T_1 - weighted images and a series of multi - slices gradient echo images pre and post the melatonin injection. The T_1 - weighted images were acquired to assure the position of the animal head in the coil, using the spin-echo sequence with a repetition delay TR = 500 ms, an echo delay TE = 15 ms and one average. The gradient echo images were acquired with TR = 600 ms, TE = 15 ms, two averages, field of view of 5 x 5 cm, matrix dimensions of 256 x 128 and 5 slices with slice thickness of 2 mm. The total acquisition time per image was 153 sec.

Twenty one multi-slices gradient echo images were acquired in each study. After the 4thimage 50 x 10^{-6}g of melatonin / kg (in saline + 7 % alcohol) was injected through the tail vein. The first four images were used as baseline. Images analysis was done by subtracting post injection images from the baseline image. The signal changes for specific brain areas were done by region of interest (ROI) analysis. To estimate the effect of the injection, the protocol was repeated with injection of saline only.

Results and Discussion

The results showed MRI responses to melatonin injection at specific brain regions of the adult and a different response in the young age groups (Fig. 1). Figure 1 shows the signal changes in different brain areas caused by the melatonin injection in the young and adult *Psammomys Obesus*. Melatonin injection in the adult *Psammomys Obesus* caused an increase in signal intensity in the frontal cortex, cingulate and striatum but not in the parietal cortex. Injection of melatonin to the young animals did not affect the signal significantly at any of the regions tested. We found, in the end of the protocol, a signal increase of 21±7 % (n=5) and -3±4 % (n=4) in the frontal cortex of adult and young, respectively (Fig.1). In the adults *Psammomys Obesus*, an increase in the BOLD contrast was generally observed 15 min post injection.

A possible explanation, is that in the young, melatonin receptors are occupied with the endogenous hormone while in the adult *Psammomys Obesus* they are free and thus may respond to exogenous melatonin. This is supported from findings of high melatonin production in young *Psammomys Obesus* at daytime. Thus, in the adult *Psammomys Obesus*, where the melatonin receptors are not occupied, the injection of melatonin causes activation of cells in certain brain areas that contain the melatonin receptors; this activation is manifested by signal increase in the gradient echo images due to the BOLD contrast. It should be noted that saline injection to adult *Psammomys Obesus* caused no signal changes.

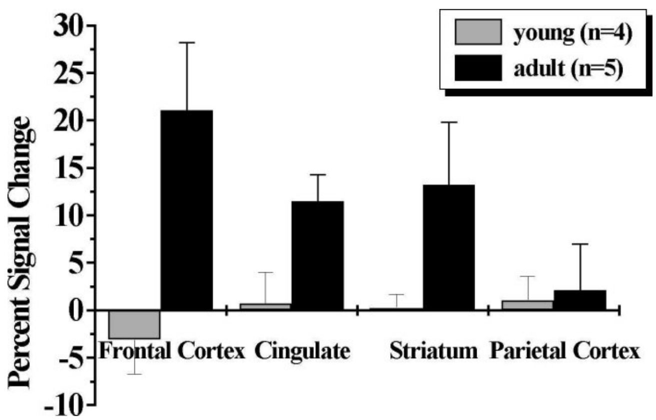

Figure 1. Bar graph showing the percentage change in the area activated by melatonin in young and adult Psammomys obesus

Conclusions

The presented ph-MRI data provides the first identification of specific brain areas that respond to melatonin. The lack of MRI response in the young may be explained by the fact that at this age the animals produce much greater amounts of melatonin having the melatonin receptors occupied.

References
(1) Chen YCI ***et al***. Magn Reson Med 1977; 38:389-398.
(2) Zhdanova IV ***et al.*** Sleep 1997; 20:899-907.
(3) Zisapel N ***et al.*** J. Exp. Zool 1999; 284:100-106.